EVALUATION OF CHROMATOGRAPHIC RETENTION DATA BY CLUSTER ANALYSIS. NEW ACHIEVEMENTS

Tibor Cserháti and Mária Szőgyi

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The newest results in the application of cluster analysis a multivariate mathematical-statistical technique for the evaluation of large retention data matrices are collected. The results are critically evaluated, and examples for the application in gas chromatography, liquid chromatographic techniques such as thin-layer chromatography and high-performance liquid chromatography, as well as in electrically driven systems are presented.

* Corresponding Authors
Fax: 06-1-3257554
E-Mail: szogyim@t-online.hu
[a] Institute of Materials and Environmental Chemistry, Research Centre for Natural Sciences, Hungarian Academy of Sciences, Budapest, Hungary

Introduction

Chromatographic techniques have been developed and employed for the separation and quantitative determination of a considerable number of organic and inorganic molecules present in complicated accompanying matrices even in very low concentrations.

The development of new mathematical-statistical computation methods for the analysis of retention data matrices has been one of the major advances in the data evaluation in up-to-date chromatographic practice. The most outstanding characteristics of this rapidly developing domain are the high-speed automated chromatographic instruments and new mathematical-statistical technologies. The evaluation of large data sets (retention characteristics of a high number of analytes measured on various stationary phase using different mobile phases, etc) is practically impossible by the well known linear regression calculation method. The modern mathematical-statistical techniques allow the parallel determination of a practically unlimited number of variables (chromatographic parameters) making possible the optimal solution of both theoretical and practical problems. Various multivariate computation methods have been successfully employed in the chromatographic practice for the identification of basic parameters influencing significantly the solute-solvent and solute-stationary phase interactions and to investigate the similarities and dissimilarities among solutes, supports and solvents. As each mathematical-statistical method highlights only several aspects of the chromatographic problem and investigation, the simultaneous employment of more than one mathematical method is rather a rule than an exception.

Cluster analysis (CA) techniques have been developed and successfully applied for the easily visualization of complicate multidimensional data matrices by reducing dimensionality. Variables with similar characteristics are near to each other on the CA dendogram, while variables with different characteristics are far away from each other. The principles of CA have been previously discussed.[1]

Gas chromatography

Foods and food products

CA has been frequently employed for the comparison of the fingerprints of food samples measured by GC-MS. Thus, the average mass scan (AMS) data obtained from essential oils were employed as variable for agglomerative hierarchical cluster analysis and principal component analysis (PCA). It was established that AMS combined with multivariate mathematical-statistic methods can be used for the analysis of essential oils.[2] The composition of steam distilled volatile oil of dried Lippia multiflora Moldenke was investigated by GC-MS and the composition was compared with those of other essential oils originated from Nigeria using CA. The calculations indicated the marked chemical variation in the species.[3] Essential oil was separated from Turkish oregano (Origanum onites L.) and its composition was analyzed by GC. CA indicated the presence of two chemo types, cravacrol and thymol. The results proved that there is no correlation between genetic structure and essential oil composition.[4] GC-combustion-isotope ratio mass spectrometry (GC-C-IRMS) has been employed for the measurement of the isotope rations of fatty acid methyl esters (FAME). The data were evaluated by linear discriminant analysis (LDA) and hierarchical CA. It was stated that the method enables the differentiation of oils according to their geographic region.[5] The effect of the grape content of musts on the composition of volatile compound was investigated by using solid-phase micro extraction (SPME) followed by GC-MS. CA calculations indicated that the change of the composition of must influences wine flavor and aroma.[6] The FAME composition of Runner peanuts were determined by GC-flame ionization detector (FID) and evaluated by CA and

factorial analysis (FA). It was established that CA separates cultivars according to the composition of FAMEs into normal, mid-, and high-oleic groups. FA computation indicated that the distribution of cultivars depends on the composition of fatty acids [7]. The fingerprints of the headspace volatile compounds of honey samples were determined by GC/MS. The data were evaluated by orthogonal partial least squares-discriminant analysis (OPLC-DA) and orthogonal partial least squares-hierarchical cluster analysis (OPLC-HCA). It was stated that the method can be employed for the botanical classification of honey samples and for the study of their chemical composition.[8] The fingerprints of Porulaca oleracea a traditional Chinese food and medicine were measured by GC/MS. The similarities and the differences among the fingerprints of different origin were computed by HCA. Computations indicated that the technique is suitable for the differentiation among the origin of samples.[9] Pyrolysis (Py) coupled to GC/MS was employed for the evaluation of the differences among the commercial samples of Cymbopogon citratus Stapf. Poaceae sold as tea. The data matrices were evaluated by HCA and PCA. Calculation indicated that the method is suitable for the correct classification of samples.[10] Fragrance alkenyl benzenes (estragole, trans-anethol, safrole, eugenol, methyl eugenol, isoeugenol, eugenyl acetate, myristicin and alpha-asarone) were separated and quantitatively determined by GC. The similarity and differences among 22 samples of essential oils were evaluated by hierarchical cluster analysis.[11]

Other analytes

GC methods combined with CA and other multivariate mathematical statistical procedures have been applied not only for the investigation of foods and food products but also in environmental protection studies, health care and biomedical research. Thus, the concentration of polycyclic aromatic hydrocarbons (PAHs) were measured in various sediments and waters in Argentina, Mediterranean sea and China. HCA was employed for the elucidation of the relationship between the amount of PAHs measured at different sampling sites.[12] The application possibilities of various mathematical statistical methods such as t-test, PCA, HCA and correlation analysis for the GC/MS metabolomics datasets have been earlier discussed.[13]

Liquid chromatographic methodologies

Thin layer chromatography

Thin-layer chromatography (TLC) is an easy to use liquid chromatographic technique. The advantages of the method are possibility of parallel determination in one run, the application of a high variation of detecting agent, and the rapidity. The disadvantages of TLC are the relative low reproducibility and moderate sensitivity. Because of the drawbacks mentioned above TLC data sets have not been frequently analyzed by CA. Reversed-phase TLC performed on octadecylsilica (ODS) layers were employed for the determination of lipophilicity of 14 s-triazine derivatives. Acetone, methanol, 2-propanol, and tetrahydrofuran were used as organic modifiers of the aqueous mobile phase. The data matrix was evaluated by PCA, HCA, partial least square (PLS) and correlation analysis.[14]

High performance liquid chromatography

Health care

HPLC has been used for the separation of the components of Prunella extracts. The experiments were motivated by the efficacy of Prunella extract to prevent and treat lung cancer. The presence of caffeic acid, rosmarinic acid, rutin, quercetin, oleanolic acid and ursolic acid in the extract was verified. Prunella samples were classified by CA. Investigations established that the efficacy of Prunella against lung cancer is attributable to multiple component acting at an optimal ratio.[15]

The anaesthetical activity of alkoxyphenylcarbamic acid esters was correlated by the calculated lipophilicity, reversed-phase HPLC retention factor and other structural parameters. The data were evaluated by PCA, CA and discriminating analysis. It was further established that artificial neural network (ANN) successfully predicted the surface anaesthetical activity.[16] The anti-inflammatory activity of the medicinal plant huang-lian has been investigated in detail. The components of the extracts of huang-lian of different origin (CEXs) were separated by HPLC and the chromatographic profile of the extracts was compared by HCA. Computations indicated that the data can be a good indicator of the biological activities of medicinal plants.[17] LC-MS was employed for the measurements of the impurities in the nerve agent precursor methylphosphonic dichloride (dichlor). HCA and factor analysis (FA were applied for the determination of the origin of the samples according to their impurity profile.[18] Polyphenols in tobacco waste were separated and identified by HPLC-PDA-ESI/MS/MS, and the concentration of chlorogenic acid and routine in the samples was measured by HPLC-UV. The data matrices were evaluated by HCA and PCA. Computations indicated that the concentration of chlorogenic acid and routine is characteristic for the tobacco wastes. It was concluded from the measurements that tobacco wastes can be used for the production of chlorogenic acid and routine.[19]

Food and food products

Various chemometrical method combined with chromatographic technologies have been frequently employed for the investigation of foods and food products controlling authenticity and origin. The use of fingerprint profiles and chemometrics for the characterization of wines has been recently reviewed.[20] The unsaponifiable fraction of virgin oils were investigated by HPLC-APCI-tandem MS. The data matrices were evaluated by PCA and CA. It was established that the method is suitable for the separation of oil cultivars according to the composition of the oil.[21]. Reversed phase HPLC (RP-HPLC) was employed for the determination of the fingerprints of 12 lentil cultivars and the data matrix was evaluated by CA.

Calculations indicated that the classification of the extracts considerable depended on the extracting agent.[22] A HPLC method was employed for the measurement of the concentration of biogenic amines in traditional Chinese sausages. It was established that the concentration of cadaverine was the highest followed by tyramine and putrescine. Samples were classified according to the profile of biogenic amines using CA. It was further found that the sanitary quality of the raw material and the specific flora exert a marked influence on the formation of biogenic amines in traditional Chinese sausages.[23]

The concentration of free amino acids during germination and seedling growth of Cola acuminata and C. anomala was measured by HPLC. PCA and CA were employed for the differentiation between the species. The computations indicated that both C. acuminata and C. anomala as well as their germination and seedling growth phases can be differentiated by the composition of free amino acids.[24]

The effect of anaerobic and aerobic conditions on the growth of Shewanella oneidensis was studied by Fourier transform infrared (FT-IR) spectroscopy and HPLC analysis of flavin compounds. The results were evaluated by CA and partial least-squares regression (PLSR). The method was proposed for the rapid characterization of the Shewanella cell metabolome in various process environments.[25] Phenolic compounds were separated and quantitatively determined in wines produced in China. Measurements were performed by HPLC-MS and the composition of the various wines was compared by HCA. Calculations indicated that the geographical parameters influence considerably the composition of phenolic compounds in wines. It was further established that the flavonol profile can be successfully applied for the discrimination between cultivars.[26]

Flavonols (myricetin, quercetin, and kaempferol) were measured in various red and white grape varieties and the similarity and differences among the chromatographic retention data were computed by HCA. It was found that the flavonol profile is suitable for the differentiation among cultivars and can be applied for chemotaxonomic aims.[27] HPLC-MS methodology was employed for the determination of the phenolic compound profiles of berry skins of nine red Vitis vinifera cultivars. The fingerprints were compared by using CA. It was established that the phenolic profile depended markedly on the type of cultivar, while the year-to-year variations were negligible.[28]

Both HPLC and GC-MS were applied for the measurement of the alkaloidal profile of Solanum nigrum complex. The data were evaluated by CA. Computation revealed significant differences among the species investigated.[29] HPLC-diode array detection (HPLC-DAD) and UPLC-DAD-TQD (ultra performance liquid chromatography) have been employed for the analysis of stilbenes. The stilbene profiles were evaluated by CA. Computations indicated that the amount of stilbenes can be enhanced by post harvest UV treatment.[30]

HPLC-PDA-MS/MS investigations were performed for the study of the composition of the tropical fruits grown in Brazil and their free scavenger activities was also measured. The relationship between the scavenger activity and chemical composition of fruits was determined by various multivariate methods such as CA and PCA. The highest level of bioactive compounds was found in buriti, otaheite apple, egg-fruit, golden spoon, physalis, piqia and star nut palm. Calculations found significant correlations between free scavenger activity and concentration of total phenolic compounds, and flavonoids.[31] The physical and chemical characteristics of pomegranate (Punica granatum L.) were investigated by various analytical such as HPLC and the distribution of the samples according to their properties was determined by CA and PCA. The results indicated the considerable differences among the pomegranate cultivars.[32] HPLC-UV was employed for the quantitative analysis of cotyledon isoflavones (genistein, daidzein, and their glucosyl and malonyl forms) and the data were evaluated by CA. Calculations proved that soybean varieties show marked differences in the composition of isoflavones and can be classified in three groups depending on the amount of isoflavones and the concentration of proteins.[33]

Plant materials

Last years the composition of medicinal herbs, the separation, quantitative determination and identification of the bioactive components have been vigorously investigated. Various chromatographic techniques such as gas chromatography, liquid chromatographic methods and electrically driven separation technologies have been frequently used for the analysis of medicinal plants.

Thus, the alkaloids in the extract of Evodia rutaecarpa (Juss.) Benth were determined by LC-ESI-MSn and the fingerprints were differentiated by various multivariate methods such as CA and PCA. Calculations established that the combined HPLC and CA method is suitable for the evaluating and controlling the quality of the extracts of E. ruteocarpa.[34] RP-HPLC combined with HCA was applied for the evaluation of the chemical fingerprinting of wild germplasm resource of Ophopogon japonicus. Computations indicated that the samples can be devided in three groups and the method allows the selection of wild germ resources of enhanced activity.[35]

A HPLC technique was applied for the measurement of the fingerprints of Keishibukuryogan formulas and the composition of the samples was compared by CA. It was stated that the method is suitable for the discrimination among samples according to the pharmaceutical manufactures, combination of ratios of botanical raw materials and time course of deterioration. The method was proposed for the quality control of multiple component drugs.[36] The fingerprints of the seed of wild Peganum harmala Linn. P. multisectum (Maxim) Bobr., P. Nigellastrum Bunge and P. variety were determined by HPLC and the chromatographic profiles were compared by PCA, HCA and linear discriminant analysis (LDA). The computations indicated that the method is suitable for the differentiation between the seeds of the different Peganum species.[37] HPLC-DAD technique was applied for the separation and quantitative determination of flavonoids in the leaves of Passiflora incarnata L., Passiforaceae. The effect of soil characteristics, environmental factors and meteorological conditions of the flavonoid content was elucidated by PCA and HCA. Calculations proved that the composition of soil, the environmental factors exert no

marked impact on the flavonoid content while the concentration of Fe, B and Cu in the soil influence considerably decreased the concentration of total flavonoids in the leaves of P. incarnata.[38]

A simple HPLC-DAD techniques was applied for the measurement of the flavonoids of sea buckthorn (TFS) and in the extract of TFS berries. The retention data were evaluated by PCA, partial least square-discriminant analysis (PLS-DA) and HCA employing Ward's minimum method of the PLS-DA loading matrix. It was concluded from the measurements that the method can be applied for the analysis of herbal extracts.[39] UPLC-DAD-FOF-MS system was applied for the measurement of the fingerprints of Cortex magnoliae officinalis species and the data were evaluated by mathematical statistical methods such as HCA, and Da. It was stated that the technique is reliable and can be employed for the quality control and authenticity test of Wen-Hou-Po.[40]

New silica-based RP-HPLC stationary phases were prepared by thermal immobilization of poly(methyloctylsiloxane) using various concentration of ligand, different times and temperatures. The separation characteristics of these RP-HPLC stationary phases were evaluated and the differences among the retention data sets was computed by HCA and PLC. Calculations proved that the separation characteristics of the new RP-HPLC stationary phases differ considerably from the majority of commercial phases.[41] The metabolic profile of Dactylopius (Hemiptera dactylopiidae) species pigments was measured by HPLC-photodiode array detector (PDA). CA and PCA were simultaneously applied for the comparison of the metabolic profiling of Dactylopius (Hemiptera dactylopiidae) species pigments according to the geographical origin and host plants.[42] HPLC-MS and GC-MS were employed for the determination of the metabolic profile of Daphnia magna.

The similarities and dissimilarities among the metabolic profiles was evaluated by CA, PCA and PLS-DA. It was stated that the method increases the amount of information obtained from aquatic toxicology testing.[43] A LC-MS/MS method was developed and applied for the discrimination and classification of Bacillus anthracis-cereus-thuringiensis strains. The data were evaluated by HCA. It was stated that the technique can be applied as an alternative method to predict similarities among microbial germs of B. cereus species without the use of whole genom sequencing.[44]

The dissolved organic fraction of a lake sediment was investigated by high-performance size-exclusion chromatography (HPSEC) and spectroscopy. The concentration of Ca, Mn, Fe, Cu, Zn and Cd was also determined. The data matrix. The data set was evaluated by CA. It was established that the calculations make possible the identification of periods with similar parameters in the lake sediment.[45] Dissolved organic matter (DOM) and dissolved organic carbon (DOC) were determined in drinking waters. HPSEC was employed for the measurement of the chromatographic profile of the samples. The similarities and differences between the water samples were elucidated by CA. It was established that the method allows the differentiation between polluted and un-polluted waters. Furthermore, the technique was proposed for the determination of the removal efficacy of coagulants (Al_2SO_4), $FeCl_3$ and high performance poly aluminium chloride.[46] Another HPSEC technology was employed for the characterization and classification of aquatic fulvic acids present in clear-water rivers and lakes. Beside HPSEC elemental analysis, liquid-state C^{13} NMR spectroscopy, and isotopic analysis were applied for the study of fulvic acids. CA and PCA were used for the classification of fulvic acids. The computations separated two clear-water groups and one brown-water group. It was established that aryl-C and O-alkyl-C content play a considerable role in the discrimination of fulvic acid species.[47]

HPSEC followed by various multivariate mathematical-statistical computation methods was applied for the investigation of cooked rice texture in relation to starch fine structure and leaching characteristics. Amylopectin fine structure was elucidated by high-performance anion-exchange chromatography coupled with pulsed amperometric detection. Correlation and stickiness of cooked rice depends considerably on the amylose/amylopectin ratio. It was established that the apparent amylose characteristics of cultivars.[48]

Hydrophilic interaction chromatography (HILIC) and reversed-phase separation mode was applied for the determination of the fingerprint of the acetonitrile-water extract of Ganoderma species a traditional Chinese medicine. The fingerprints obtained on various columns were differentiated by HCA. It was concluded from the data that the simultaneous application of fingerprints measured in different chromatographic conditions increases considerably the reliability of the analysis.[49]

Electrically driven systems

The high separation power, and relatively simple instrumentation of capillary electrophoresis (CE) and related technologies facilitated their successful application in many fields of up-to-date scientific research such as health care, analysis of pharmaceutical, foods and food products, etc. Similarly to other chromatographic methodologies CA has also found application in the elucidation of CE migration time data. Thus, CE was employed for the determination of the nucleoside and modified nucleoside profiles of urogenic tract cancer patients and healthy controls. The data matrix was evaluated by various multivariate mathematical-statistical methods such a PCA, HCA, K-nearest neighbor method (kNN) and partial least squares-discriminant analysis (p-PLS-DA). Computations proved that the sensitivity and specificity of the method were 76.5% and 80.2%. It was stated that the fingerprints of urinary nucleosides can be used as a reliable and convenient tool for the diagnostic of urogenital cancer diseases.[50] Proteomic approaches were employed for the identification of altered proteins in endometrial carcinoma. The objectives of the measurements were the search for potential biomarkers or therapeutic targets. Proteins were extracted and separated by 2-dimensional electrophoresis. The method identified 99 proteins. CA established that the proteins are involved in various biochemical processes. It was concluded that the

measurements may promote the study of endometrial carcinogenesis, investigation of the spatial and genotypic clustering of Salmonella. Genetic similarities were evaluated by CA. Computation indicated the relative homogeneity of isolates.[51, 52] Capillary electrophoresis fragment sizing system was applied for the identification of the intra-varietal diversity within 'Askari' and 'Keshmesh' (Vitis vinifera L.). The similarity of samples were investigated by CA. It was stated that the method can be applied for the identification of intra-cultivar diversity.[53] Cellulose-acetate electrophoresis was used for the isozyme and protein separation from the mycelial extracts of 27 isolates of Trichoderma harzianum, 10 isolates of T. aureoviride, and 10 isolates of T. Longibrachiatum. The relationship among the isolates was revealed by CA. The results showed that the distance between T. harzianum and T. aureoviride is smaller then to T. longibrachiatum. It was further established that T. harzianum isolates show the highest genetic variation.[54]

Hydrophobic interaction chromatography (HIC) before 2-dimensional gel elecrophoresis (2DGE) was employed to remove highly abundant proteins of plasma which influence the detectability of low abundance proteins of biomedical interest. CA was applied for the classification of plasma proteins according to their hydrophobicity (low, medium and high). The depleting capacity of HIC was compared with that of immuno-affinity (IA) column. It was suggested that HIC can be applied as an alternative procedure to IA.[55] The effect of plant growth (Ryegrass, Lolium perenne) and microbial strains (Bacillus subtilis, Sphingobacterium multivolume, acinetobacter radioresistens, Rhodococcus erythropolis, and Pseudomonas fluorescence) on the soil petroleum remediation was investigated. Denaturing gradient gel electrophoresis (DGGE) measurements suggested that the best results can be achieved by the simultaneous application of plant growth and complex microbial community. The differences between the treatments were evaluated by CA.[56]Thin-layer chromatography, CE, FTIR spectroscopy, ICP-TOF-MS (inductively coupled plasma time-of flight mass spectrometry) with laser ablation were evaluated by CA and PCA. The method was proposed for the rapid comparative analysis of unknown samples.[57]

CA has also found application for the evaluation of micellar electrokinetic chromatography data. Samples were separated by an electrolyte consisting of 100 mM borate (pH 9.8) and 20 mM sodium dodecylsulfate. Tea infusions were injected for 5 s at 0.5 psi. The total length of the fused silica capillary was 60 cm, the internal diameter being 75 µm. Analytes were detected by a laser-induced fluorescence detector. Green teas were differentiated by CA.[58]

Abbrevations

AMS	average mass scan,
CA	cluster analysis,
FA	factorial analysis,
FAME	fatty acid methyl ester,
FID	flame ionization detector,
GC-C-IRMS	gas-chromatography-combustion-isotope ratio mass spectrometry,
HCA	hierarchical cluster analysis,
OPLC-DA	orthogonal partial least squares-discriminant analysis,
OPLC-HCA	orthogonal partial least squares-hierarchical cluster analysis,
PCA	principal component analysis,
SPME	solid-phase microextraction.

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